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Medicinal plants, chemical composition and quality: may blackcurrant buds and blackberry sprouts be a new polyphenol source for herbal preparations?

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Summary

It is well known that plants are important sources for the preparation of natural remedies as they contain many biologically active compounds: in particular, phenolic compounds are one of the most widely occurring groups of phytochemicals. Some endemic species may be used for the production of herbal preparations containing phytochemicals with significant antioxidant capacities and health benefits: blackberry sprouts and blackcurrant buds are known to contain appreciable levels of phenolic compounds, including flavonols, phenolic acids and catechins.

The aim of this research was to perform an analytical study of blackcurrant and blackberry bud-preparations, in order to identify the main bioactive polyphenolic compounds, to study the total polyphenolic content and to obtain a specific profile of the main polyphenols contained in these products using a High Performance Liquid Chromatograph – Diode Array Detector; the same analyses were performed both on the University lab preparations and on commercial preparations.

Different chromatographic methods were used to determine concentrations of phytochemical compounds in the preparations, allowing for quantification of statistically significant differences in their polyphenolic content both in the case of *Ribes nigrum* and *Rubus ulmifolius*.

The assessment of chemical composition and bioactivities of the plant-derived products could help in find out new sources of natural antioxidants and other health-promoting compounds: only with the deep knowledge of the bioactive composition of plant preparations it will be possible to develop a new generation of standardized, effect-optimized, mono- and multi-extract preparations.

Introduction

Plants are important sources for the preparation of natural remedies, food additives, and other ingredients, as they contain many biologically active compounds as polyphenols, vitamins (A, B_6, C, E) and other very important phytochemicals. In particular, phenolic compounds are one of the most widely occurring groups of phytochemicals (Dvaranauskaite et al., 2008; Komes et al., 2011). In the plant kingdom, bioactive polyphenolic compounds can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. (Tabart et al., 2011; Tabart et al., 2012). These low molecular weight secondary plant metabolites exhibit excellent antioxidant properties. However, their particular mechanisms of action vary depending both on the structure and environment (Komes et al., 2011).

There is increasing evidence in the literature which indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important. Among the various medicinal and culinary herbs, some phenolics are ubiquitous compounds found in all plants as secondary metabolites (Rababah et al., 2011; Mattila et al., 2011; Sakakibara et al., 2003). Some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits in the prevention of chronic diseases such as cancer, cardiovascular and neurodegenerative diseases (Mattila et al., 2011; Donno et al., 2012b; Canterino et al., 2012); however, polyphenols may also have a negative effect on health because they form complexes with iron and therefore reduce the bioavailability of this essential element (Santos-Buelga and Scalbert, 2000; Donno et al., 2012a; Moller et al., 2009). Positive therapeutic effects may be related to their antioxidant activity as well their ability to regulate cellular activities of inflammation-related cells (Mattila et al., 2011; Tabart et al., 2012).

However, since more than 5000 phytochemicals have been identified and different antiproliferation mechanisms have been reported, it is believed that synergistic or additive biological effects of multiple phytochemicals, rather than a single compound or a group of compounds, contribute to disease prevention. In this context, the diffusion and the use of herbal preparations to prevent some common diseases could be interesting (Jia et al., 2012).

The use of plant extracts as functional ingredients in various foods, medical and cosmetic applications is gaining growing interest among scientists, as well as among consumers and food manufacturers (Komes et al., 2011).

A variety of berries have been demonstrated to exhibit a broad spectrum of benefits: in particular, blackberry sprouts and blackcurrant buds are known to contain appreciable levels of phenolic compounds, including flavonols, phenolic acids and catechins (Lugasi et al., 2011; Cho et al., 2005). Blackcurrant (*Ribes nigrum* L.) is a shrub spontaneously growing in the cold and temperate climatic zones. The most important industrial product of black currant is fruits; however, leaves and buds, due to their characteristic chemical composition and excellent flavor, have also found some applications as a raw material for the herbal and cosmetic industries: many people use its buds as medicinal preparation for its anti-inflammatory activity and anti-dermal diseases (eczema and psoriasis) (Dvaranauskaite et al., 2008).

Blackberry (*Rubus ulmifolius* Schott) is a Rosaceae plant that grows in shrubs in temperate regions worldwide and the main importer is U.S.A., which is supplied from Chile, Costa Rica, Guatemala and Mexico: sprouts have been used in traditional medicine for their many medicinal properties, as anti-inflammatory activity and anti-haemorrhoids and diarrhoea activity (Gudej and Tomczyk, 2004; Zuniga-Hansen et al., 2010).

In particular, bud and sprout preparations, derived from embryonic fresh plant tissues, are important therapeutic remedies, prescribed in hepatic, respiratory, circulatory and inflammatory disorders, but data on their chemical composition are lacking as, until now, phytochemical studies have principally been performed on barks, roots and root exudates, leaves, fruits and seeds (Donno et al., 2012a; Peev et al., 2007).

The aim of this research was to perform an analytical study of blackcurrant and blackberry bud-preparations based on different genotypes, in order to identify the main bioactive polyphenolic

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compounds, to quantify the total polyphenolic content and to obtain a specific profile of the main polyphenols contained in these products using a High Performance Liquid Chromatograph – Diode Array Detector; the same analyses were performed both on University lab preparations and on commercial preparations.

Materials and methods

Plant material

Samples of analysed species, *Ribes nigrum* L. and *Rubus ulmifolius* Schott, were picked up in two years (2011 and 2012), in February (blackcurrant buds) and in May (blackberry young sprouts), in two germplasm repositories in Turin Province (Italy), Grugliasco (*Rubus ulmifolius*) and San Secondo di Pinerolo (*Ribes nigrum*). Among the chosen species, different varieties were sampled, in order to test the genotype effect on the chemical composition of the final product (black currant: Rozenthal and Daniels; blackberry: Kiowa, Nightfall and a wild variety). Buds and sprouts were used fresh to prepare herbal preparations; HPLC samples were analysed after being stored for a few days at normal atmosphere (N.A.), at 4°C and 95% relative humidity (R.H.).

Commercial products from two different Italian herbal companies were also analysed to compare their quality and to understand the effect of the utilization of different local plant materials on the final quality of the product: the two companies are located in San Gregorio di Catania (Catania Province, Company 1), and Predappio (Forlì-Cesena Province, Company 2). Tab. 1 shows the genotypes, the sampling times and the picking sites of analyzed herbal preparations (University and Company preparations).

Macerated sample preparation protocol

The protocol of bud-preparations is detailed in the monograph "Homeopathic preparations", quoted in the French Pharmacopoeia, 8th edition, 1965 (PHARMACIENS, 1965). Bioactive compounds were extracted through a cold maceration process for 21 days, in a special solution of ethanol (95%) and glycerol, followed by a first filtration (Whatman Filter Paper, Hardened Ashless Circles, 185 mm Ø), a manual pressing and, after two days of decanting, a second filtration (Whatman Filter Paper, Hardened Ashless Circles, 185 mm Ø). Macerated samples were then stored at N.A., at 4°C and 95% R.H.

Calibration standards

All calibration standards were purchased from Sigma Aldrich (USA): chlorogenic acid, caffeic acid, ferulic acid, hyperoside, isoquercitrin, quercitrin, quercetin, gallic acid, ellagic acid, catechin and epicatechin. Fig. 1 shows the chemical structures of the all detected polyphenolic compounds, divided by classes.

Quantitative determinations were performed using an external standard method. Calibration curves in the 125 - 1000 mg/L range with good linearity ($R^2 > 0.998$) for a four point plot were used to determine the concentration of polyphenolic compounds in bud-preparation samples. Stock solutions of cinnamic acids and flavonols with a concentration of 1.0 mg/mL were prepared in methanol: from these solutions, four calibration standards were prepared by dilution with methanol; stock solutions of benzoic acids and catechins with a concentration of 1.0 mg/mL were prepared in 95% methanol and 5% water: from these solutions, four calibration standards were prepared by dilution with 50% methanol-water.

HPLC analysis

An Agilent 1200 High Performance Liquid Chromatograph, equipped with a G1311A quaternary pump, a manual injection valve and a 20 µl sample loop, coupled to an Agilent GI315D UV-Vis diode array detector, was used for the analysis.

The maceration solvents, ethanol and glycerol, were purchased from Fluka Biochemika (Switzerland) and Sigma Aldrich (USA) respectively. Analytic HPLC grade solvents, methanol and formic

Tab. 1: Species, genotype, sampling time and picking site of the analysed buds.

University bud-preparations			
Species	**Genotype**	**Year**	**Germplasm repository**
Ribes nigrum L.	Rozenthal	2011	San Secondo di Pinerolo
		2012	
	Daniels	2011	San Secondo di Pinerolo
		2012	
Rubus ulmifolius Schott	Nightfall	2011	Grugliasco
		2012	
	Kiowa	2011	Grugliasco
		2012	
	Wild variety	2011	Grugliasco
		2012	
Company bud-preparations			
Species	**Company**	**Year**	**Germplasm repository**
Ribes nigrum L.	Company 1	2011	San Gregorio di Catania
	Company 2	2011	Predappio
Rubus ulmifolius Schott	Company 1	2011	San Gregorio di Catania
	Company 2	2011	Predappio

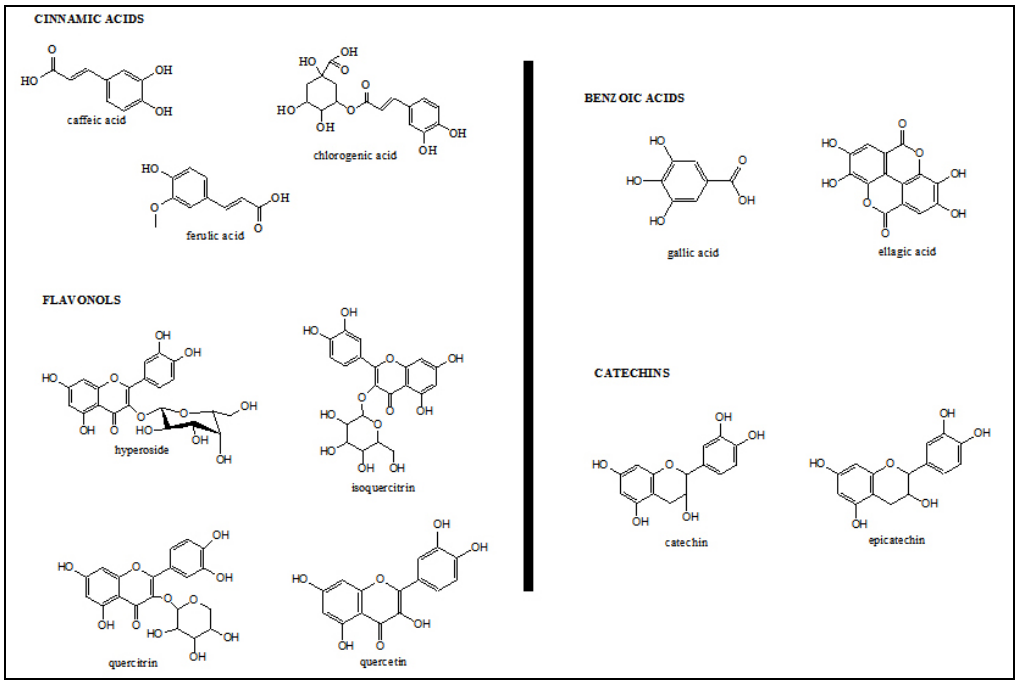

Fig. 1: Chemical structures of the detected polyphenolic compounds.

acid, were purchased from Sigma Aldrich (USA) and Fluka Biochemika (Switzerland) respectively; potassium dihydrogen phosphate was also purchased from Sigma Aldrich (USA).

All samples were analysed in triplicate (three repetitions for three plants for each sample), and all data are given in order to assess the repeatability of the used methods (standard deviation).

Two different chromatographic methods were used to analyse the macerated samples. The first method (A) was used for the analysis of cinnamic acids and flavonols; bioactive compound separation was achieved on a ZORBAX Eclipse XDB – C18 column (4.6 x 150 mm, 5 µm), while the mobile phase consisted of methanol and a solution of 40 mM potassium dihydrogen phosphate in water. The flow rate was 1.0 mL min^{-1} (gradient analysis, 60 minutes) and the detector wavelength was 330 nm (Peev et al., 2007; Donno et al., 2012a). The second method (B) was used for the analysis of benzoic acids and catechins; bioactive molecules were separated on a ZORBAX Eclipse XDB – C18 column (4.6 x 150 mm, 5 µm), while the mobile phase consisted of a solution of methanol/water/formic acid (5:95:0,1 v/v/v) and a mix of methanol/formic acid (100:0,1 v/v). The flow rate was 1.0 mL min^{-1} (gradient analysis, 35 minutes) and the detector wavelengths were 250, 280 and 320 nm (Donno et al., 2012a; Moller et al., 2009).

The detection limits (DL) and the quantification limits (QL) of the two chromatographic methods were calculated as the minimal concentration producing a reproducible peak with a signal-to-noise ratio greater than 3 and 10, respectively; Tab. 2 shows the DL and the QL of the all considered compounds. Accuracy was checked by spiking samples with a solution containing each phenolic compound in a concentration of 10 mg/mL.

Tab. 2: Detection limits (DL) and quantification limits (QL) of the two used chromatographic methods for each calibration standard

Standard	Analysis method	DL (mg/L)	QL (mg/L)
caffeic acid	method A	1.23	4.11
chlorogenic acid		0.63	2.09
ferulic acid		1.01	3.37
hyperoside		0.55	1.83
isoquercitrin		0.48	1.58
quercitrin		1.07	3.57
quercetin		1.90	6.32
gallic acid	method B	0.28	0.94
ellagic acid		1.88	6.27
catechin		1.75	5.85
epicatechin		1.75	5.83

Identification of polyphenolic compounds

All single compounds were identified in samples by comparison of their retention times and UV spectra with those of standards in the same chromatographic conditions. Total polyphenolic compounds (TPC) were determined as the sum of the most important classes of polyphenols present in the samples. Four polyphenolic classes were considered: benzoic acids (gallic acid and ellagic acid), catechins (catechin and epicatechin), cinnamic acids (chlorogenic acid, caffeic acid and ferulic acid) and flavonols (hyperoside, isoquercitrin, quercitrin and quercetin). All results were expressed as mg per 100 g of buds fresh weight (FW).

Statistical Analysis

Results were subjected to ANOVA and t Student Test for mean comparison (SPSS 18.0 Software) and HSD Tukey multiple range test ($P<0.05$). Principal Component Analysis (PCA) was performed on the single polyphenolic concentration data.

Results

Total polyphenolic content

Ribes nigrum L.

Regarding *Ribes nigrum*, statistically significant differences were observed between the two varieties in both years, with a minimum TPC value of 427mg/100 g_{FW} (Rozenthal 2011) and a maximum of 834mg/100 g_{FW} (Daniels 2012); there were also significant differences in TPC in the same variety in two different years. In the commercial bud-preparations, there were statistically significant differences between the products of the two different companies with a maximum value of 856mg/100 g_{FW} (Company 2), a result similar to the mean value of cv Daniels (2012 year) (Fig. 2).

Rubus ulmifolius Schott

In the case of *Rubus ulmifolius*, two statistically different groups were observed: the first one with four samples (Kiowa 2011, Kiowa 2012, wild variety 2011 and wild variety 2012) and the second one with two samples (Nightfall 2011 and Nightfall 2012); statistically significant differences were not observed between the two years in each analysed genotype: TPC ranged from a value of 211 mg/100 g_{FW} (Kiowa, 2012) to a value of 276 mg/100 g_{FW} (Nightfall, 2011). Regarding the commercial bud-preparations, results showed statistically significant differences between the two companies with a maximum value of 384 mg/100 g_{FW} (Company 1), about 30 mg/100 g_{FW} higher than Company 2 (Fig. 3).

Single polyphenolic profiles

All data are reported in Tab. 3 (blackcurrant buds) and 4 (blackberry sprouts).

Ribes nigrum L.

Blackcurrant samples showed the following polyphenolic composition: three cinnamic acids (chlorogenic acid, caffeic acid, ferulic acid), one flavonol (quercetin), one benzoic acid (gallic acid) and two catechins (catechin, epicatechin); in the cultivar Daniels (2011 and 2012 years) chlorogenic acid was not detected. Single polyphenolic concentration ranged from 4 mg/100 g_{FW} (chlorogenic acid, Rozenthal 2012) to 292 mg/100 g_{FW} (catechin, Daniels 2012) (Fig. 4). Statistically significant differences were observed among the University bud-preparation samples, while in commercial bud-preparations there were significant differences only in catechin and epicatechin.

Multivariate analysis

Principal Component Analysis reduced variables into two principal components (82.78% of total variance) and divided samples in four groups, as the initial sample groups, confirming the statistically significant differences of ANOVA Test on TPC. PCA variable graph showed a correlation among quercetin, ferulic acid, gallic acid and chlorogenic acid to PC1, while caffeic acid, catechin and epicatechin were in an intermediate position between PC1 and PC2 (Fig. 5).

Rubus ulmifolius Schott

Blackberry samples showed the following polyphenolic composition: two cinnamic acids (caffeic acid, ferulic acid), three flavonols (hyperoside, isoquercitrin, quercitrin) and two benzoic acids (gallic acid, ellagic acid): single polyphenolic concentration ranged from 5 mg/100 g_{FW} (isoquercitrin, wild variety 2012) to 92 mg/100 g_{FW}

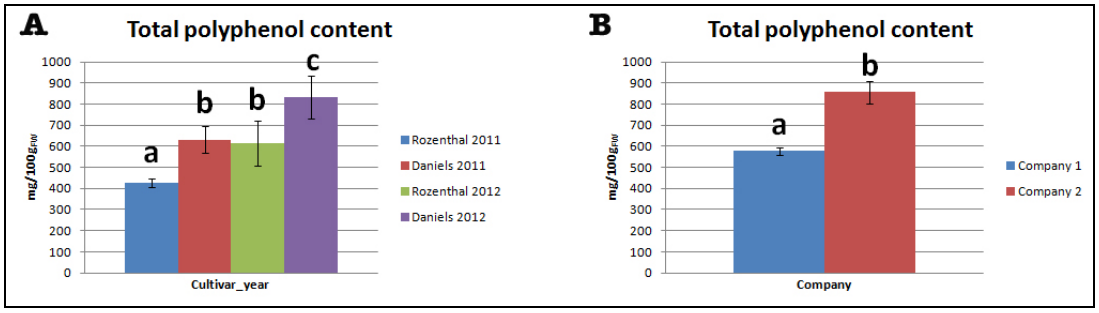

Fig. 2: *Ribes nigrum*: TPC in University bud-preparations (A) and Company bud-preparations (B). Different letters for each sample indicate the significant differences at P<0.05.

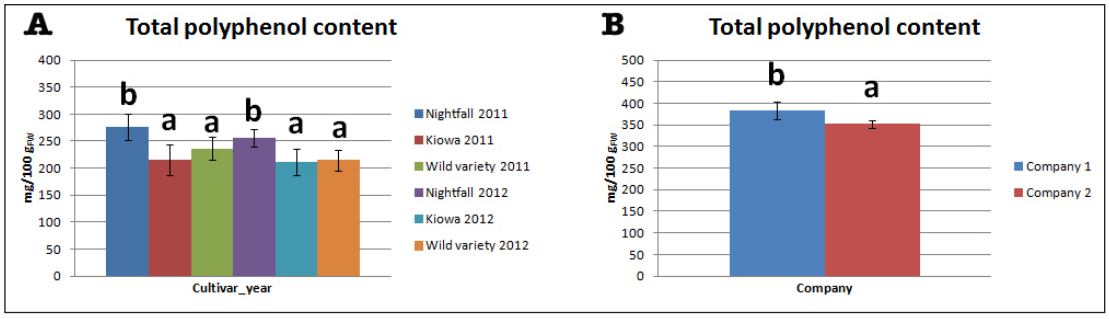

Fig. 3: *Rubus ulmifolius*: TPC in University bud-preparations (A) and Company bud-preparations (B). Different letters for each sample indicate the significant differences at P<0.05.

Tab. 3: *Ribes nigrum*: single polyphenolic profile in University bud-preparations and commercial bud-preparations. Different letters for each sample indicate the significant differences at P<0.05.

METHOD A	*cinnamic acids*									*flavonols*											
	(mg/100 gFW)									*(mg/100 gFW)*											
sample name	*chlorogenic acid*			*caffeic acid*			*ferulic acid*			*hyperoside*			*isoquercitrin*			*quercitrin*			*quercetin*		
Ribes nigrum L.	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*
Rozenthal 2011	6,14	b	1,56	7,02	ab	0,63	127,51	a	10,72	nd	/	/	nd	/	/	nd	/	/	92,43	a	5,99
Daniels 2011	nd	/	/	12,73	c	2,75	200,82	b	64,97	nd	/	/	nd	/	/	nd	/	/	121,66	c	11,49
Rozenthal 2012	3,94	a	1,43	6,07	a	1,22	128,17	a	26,21	nd	/	/	nd	/	/	nd	/	/	106,28	b	13,18
Daniels 2012	nd	/	/	8,68	b	1,00	205,15	b	14,67	nd	/	/	nd	/	/	nd	/	/	125,64	c	11,31
Company bud-preparations	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*
Company 1_Blackcurrant	3,50	a	1,17	8,04	a	1,67	153,03	b	11,28	nd	/	/	nd	/	/	nd	/	/	124,03	a	8,75
Company 2_Blackcurrant	3,72	a	1,29	8,78	a	1,19	177,88	b	9,81	nd	/	/	nd	/	/	nd	/	/	123,95	a	17,34
METHOD B	*benzoic acids*						*catechins*														
	(mg/100 gFW)						*(mg/100 gFW)*														
sample name	*gallic acid*			*ellagic acid*			*catechin*			*epicatechin*											
Ribes nigrum L.	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*									
Rozenthal 2011	16,72	a	1,77	nd	/	/	121,01	a	9,42	56,05	a	4,49									
Daniels 2011	62,19	b	5,93	nd	/	/	179,21	b	5,78	55,70	a	3,12									
Rozenthal 2012	20,42	a	3,33	nd	/	/	244,87	c	62,37	105,60	b	26,61									
Daniels 2012	61,13	b	7,27	nd	/	/	291,78	c	55,65	141,57	c	18,26									
Company bud-preparations	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*									
Company 1_Blackcurrant	34,88	c	8,12	nd	/	/	162,56	a	9,79	92,21	a	10,30									
Company 2_Blackcurrant	34,03	c	4,65	nd	/	/	318,23	b	52,75	189,74	b	7,02									

Tab. 4: *Rubus ulmifolius*: single polyphenolic profile in University bud-preparations and commercial bud-preparations. Different letters for each sample indicate the significant differences at P<0.05.

METHOD A	*cinnamic acids*									*flavonols*												
	(mg/100 gFW)									*(mg/100 gFW)*												
sample name	*chlorogenic acid*			*caffeic acid*			*ferulic acid*			*hyperoside*			*isoquercitrin*			*quercitrin*			*quercetin*		
Rubus ulmifolius Schott	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*
Nightfall 2011	nd	/	/	7,42	ab	2,39	83,37	c	4,40	33,88	c	2,85	35,76	b	4,41	19,71	a	2,99	nd	/	/
Kiowa 2011	nd	/	/	10,84	b	1,77	52,60	b	10,90	26,00	ab	4,53	8,24	a	1,83	20,61	a	3,34	nd	/	/
Wild variety 2011	nd	/	/	21,27	c	3,58	33,20	a	6,17	27,49	b	3,12	5,86	a	0,87	31,91	b	6,27	nd	/	/
Nightfall 2012	nd	/	/	6,16	a	0,82	86,33	c	3,86	35,06	c	3,96	34,01	b	3,53	20,26	a	2,87	nd	/	/
Kiowa 2012	nd	/	/	9,74	ab	1,36	49,44	b	5,66	21,66	a	2,54	8,06	a	1,13	23,13	a	2,40	nd	/	/
Wild variety 2012	nd	/	/	22,78	c	4,16	37,98	a	4,46	24,97	ab	4,17	5,03	a	1,25	33,08	b	3,98	nd	/	/
Company bud-preparations	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*
Company 1_Blackberry	nd	/	/	20,06	b	3,90	42,65	a	4,07	23,79	a	1,00	6,97	a	1,80	35,30	b	4,17	nd	/	/
Company 2_Blackberry	nd	/	/	22,20	b	2,78	38,77	a	1,76	30,88	b	4,00	6,89	a	1,52	28,36	a	2,44	nd	/	/
METHOD B	*benzoic acids*						*catechins*														
	(mg/100 gFW)						*(mg/100 gFW)*														
sample name	*gallic acid*			*ellagic acid*			*catechin*			*epicatechin*											
Rubus ulmifolius Schott	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*									
Nightfall 2011	17,13	ab	12,36	78,31	bc	13,04	nd	/	/	nd	/	/									
Kiowa 2011	5,52	a	1,75	91,60	c	23,49	nd	/	/	nd	/	/									
Wild variety 2011	46,54	d	20,50	70,37	abc	18,98	nd	/	/	nd	/	/									
Nightfall 2012	19,92	abc	2,57	54,28	a	10,23	nd	/	/	nd	/	/									
Kiowa 2012	33,11	cd	10,83	66,33	ab	11,51	nd	/	/	nd	/	/									
Wild variety 2012	24,15	bc	6,64	67,06	ab	13,53	nd	/	/	nd	/	/									
Company bud-preparations	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*	*mean*	*Tukey HSD*	*SD*									
Company 1_Blackberry	26,16	b	4,70	229,49	b	19,88	nd	/	/	nd	/	/									
Company 2_Blackberry	15,81	a	1,89	208,90	a	8,67	nd	/	/	nd	/	/									

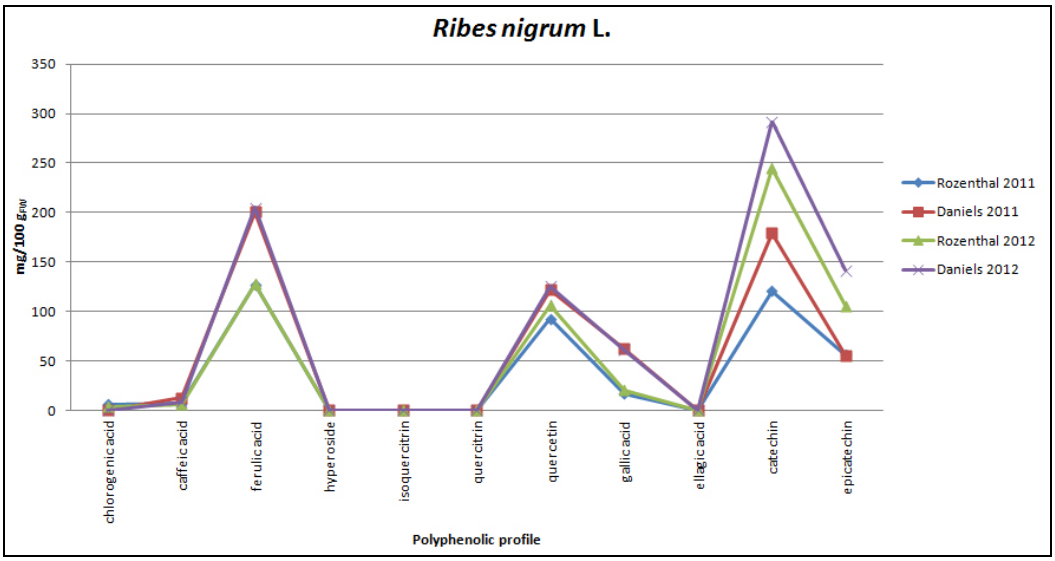

Fig. 4: *Ribes nigrum*: single polyphenolic profiles in University bud-preparations.

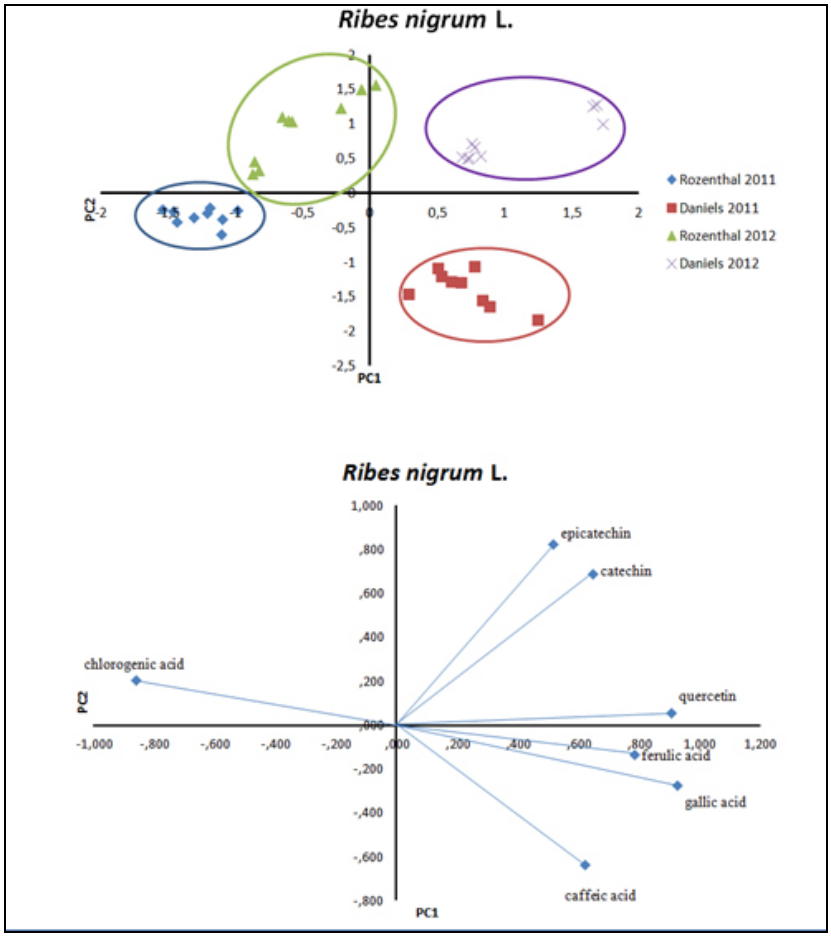

Fig. 5: *Ribes nigrum*: PCA individual and variable graph of University bud-preparation samples.

(ellagic acid, Kiowa 2011) (Fig. 6). There were statistically significant differences among the University and company bud-preparations samples, but in this second case differences were not observed in cinnamic acids and isoquercitrin.

Multivariate analysis

Principal Component Analysis was performed on all samples and it reduced the initial six groups into three groups overlapped with the genotypes: the Kiowa and wild variety groups confirmed

very similar TPC ANOVA results. PCA variable graph showed a correlation between cinnamic acids and flavonols to PC1 (54.91% of total variance) and a correlation between ellagic acid and PC2 (19.28% of total variance); gallic acid was in an intermediate position between PC1 and PC2 (Fig. 7).

Commercial bud-preparations

In Fig. 8 single polyphenolic profiles showed a similar fitting between the two company bud-preparations, both in blackcurrant and blackberry: there was a substantial difference only in blackcurrant samples, with the Company 2 showing an higher catechin value than Company 1.

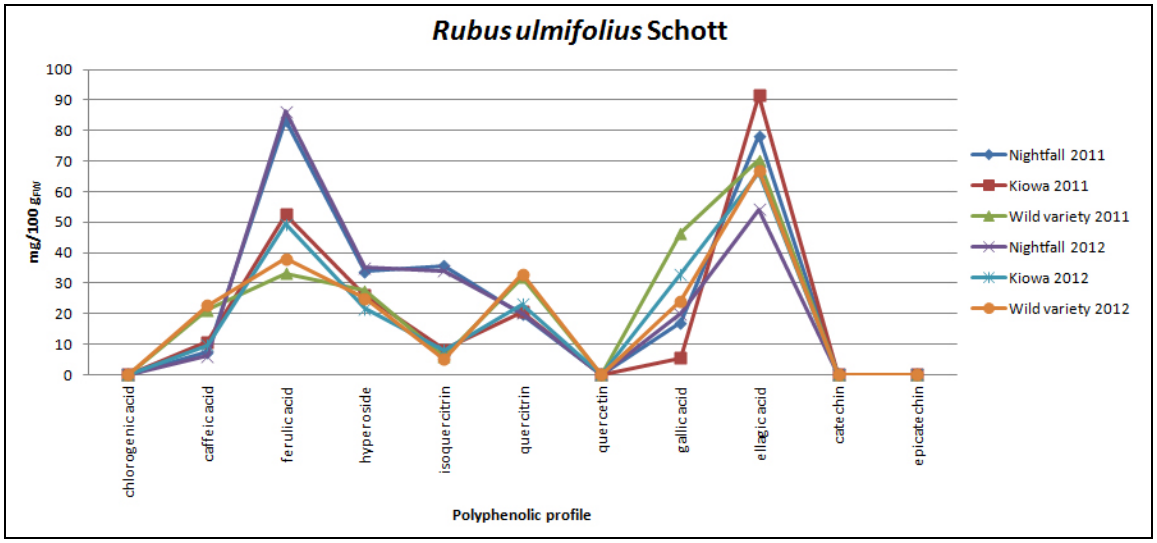

Fig. 6: *Rubus ulmifolius*: single polyphenolic profiles in University bud-preparations.

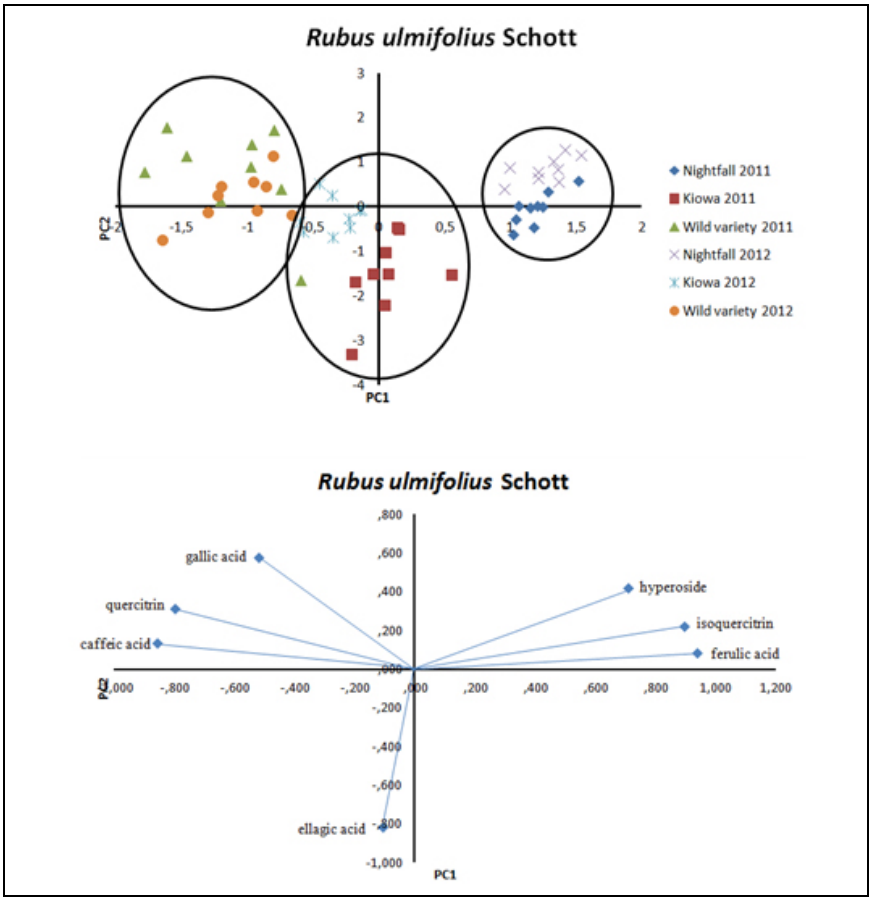

Fig. 7: *Rubus ulmifolius*: PCA individual and variable graph of University bud-preparation samples.

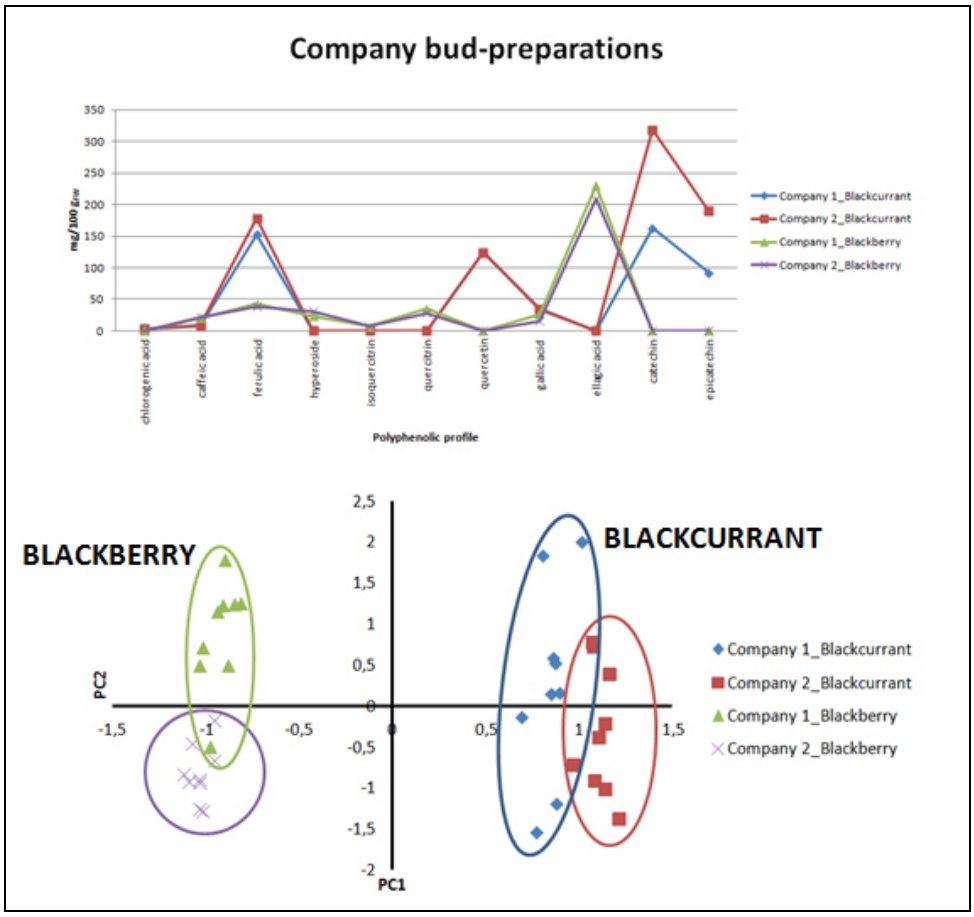

Fig. 8: Company bud-preparations: single polyphenolic profiles and PCA individual graph.

Multivariate analysis

PCA were performed on company samples and two PC were extracted with a variance value of 92.85% of initial one: PCA individual graph showed four groups similar to initial groups, but the Company 1 and the Company 2 data were in two very near groups; this consideration was observed both in blackcurrant and blackberry (Fig. 8).

Discussion

In the last few decades, many screening studies of different plant materials have been performed in order to find naturally occurring antioxidants for use in food or medicinal preparations, as replacements for potentially harmful synthetic additives (Komes et al., 2011).

It was reported that extracts from berries processing byproducts contained a high amount of phenolic compounds and possessed remarkable antioxidant activity (Dvaranauskaite et al., 2008). The information on antioxidant properties of blackcurrant buds and blackberry sprouts is rather scarce. Recently it was reported that buds and sprouts had an higher content in phenolics and antioxidants than fully ripened berries (Peev et al., 2007; Donno et al., 2012a; Dvaranauskaite et al., 2008).

Reports on the analysis of phenolic acids (e.g. caffeic acid and its derivatives) by HPLC coupled to diode array or mass detectors have been published. They describe phenolic acid determination in medicinal plants and preparations, as blackcurrant and blackberry bud-preparations (Urpi-Sarda et al., 2009; Castro et al., 2010), according to single bioactive compound concentrations showed in this research. Among other identified classes, flavonols and catechins were also selected for quantitative studies (Huang et al., 2008; Surveswaran et al., 2010; Yi et al., 2009).

The bud-preparations of the species analysed in this work are recommended by physicians to be consumed as polyphenol supplements, and further information on these compounds could be used to direct future research towards condition-specific beneficial properties associated with their therapeutic effects. Berry plants with high levels of polyphenolic compounds are sought-after, especially if they have a long history of regular use that attests to their safety (Donno et al., 2012a). As showed in this research, improvement of the raw plant material may be achieved by plant breeding and selection and the levels of bioactives can be increased to consistently high levels (Joubert et al., 2008).

It is well-known that chemical composition of secondary plant metabolites highly depends on such factors as climatic conditions, harvesting time, and plant genotype (Dvaranauskaite et al., 2008; Donno et al., 2012a) and the results of this research confirm this hypothesis: different species and varieties present different composition and concentrations of polyphenolic compounds, but it is also important to consider pedoclimatic characteristics of sampling sites strongly influence the presence of these molecules, as comparing the results of commercial bud-preparations. The results are highly variable depending on the genera: in *Ribes nigrum* the sampling year influences the TPC of analysed varieties, while in *Rubus ulmifolius* there are not differences between two years for each cultivar.

ANOVA and PCA results showed that the analyzed bud-preparation composition was similar in all the samples but the single compound concentrations were different; moreover, observing the chemical composition, results showed that few compounds were not detected in herbal medicines: chromatographic bioactive compound profiles could be applied in the differentiation of specific bud-preparations by other species (DONNO et al., 2012a; ZHAO et al., 2009).

Regarding chromatographic analysis, many good HPLC methods exist for the separation and quantification of different polyphenolic groups found in various plant materials: in this research used methods showed that a good separation could be achieved by using common HPLC solvents as mobile phase (methanol, water, formic acid, potassium dihydrogen phosphate solutions). The methods were sensitive and selective by using multiple wavelengths corresponding to the different UV-Vis maximum absorptions of the different polyphenolic groups in buds and sprouts. The results indicated that the developed methods were feasible for comprehensive authentication and quality control of bud-preparations.

Certain polyphenolics can be used collectively as representative standards of a plant sample in quantification (TSAO and YANG, 2003), as done in this study, but these methods still allow quantification of individual compounds; such HPLC data can be used as TPC for the quantification of bud or sprout phenolics. HPLC methods give more information on individual compounds or groups of compounds (TPC) than the TPC by Folin-Ciocalteu method (GUDEJ and TOMCZYK, 2004).

Knowledge of polyphenols molecular structure, composition and quantity is necessary to understand their role in determining potential health effects (HAGER et al., 2008): this study is only a preliminary research about bud-preparation chemical composition of two berryfruit species, in order to detect the most important polyphenolic classes and single compounds, but a further quantitative evaluation on the basis of their native structures with HPLC coupled to mass spectrophotometry is necessary.

Conclusions

The assessment of chemical composition and bioactivities of the plant-derived products could help in find out new sources of natural antioxidants and other health-promoting compounds which could be used as natural remedies, food additives, functional food and nutraceutical ingredients. Due to the differences in the raw material, extraction and analysis methods, the data regarding the phenolic content of different plant species and genotypes is difficult to compare (DVARANAUSKAITE et al., 2008): only with the deep knowledge of the bioactive composition of plant preparations it will be possible to develop a new generation of standardized, effect-optimized, mono- and multi-extract preparations which fulfill today's standards for quality, safety and efficiency of medicinal drugs (DVARANAUSKAITE et al., 2008; KOMES et al., 2011).

Further studies should be focused on the quantification of antioxidant activity of detected bioactive compounds in the extracts as well as on the possibilities to use natural antioxidants from berry buds and sprouts for different applications, which are likely to improve commercial use of these plants.

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